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ON A NEW SPECIES OF *CLAVELINA* FROM JAPAN,
WITH REMARKS ON ITS MODE OF BUDDING^{1,2)}

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With Text-figures 1-3

Colonies of a strange form of the Clavelininae were found by Mr. Hirokazu Fujimoto of the Faculty of Science, Tokyo Kyoiku University on the eastern rocky shore at Tatado, west of Shimoda, during the spring tide in May, 1972. The colony varied considerably in size, always being intertwined with other ascidians, hydroids and small algae. Thus, it was very difficult to see the exact range of respective colonies, though the colonies themselves were distinguishable in the field by the bright orange colouration of completely exposed thoraces. Since then, the colonies have been reared continuously by the first author on glass-plates set immersed in the inlet near the Shimoda Marine Biological Station, and it was found that this animal performs a unique type of budding and never forms any real colonies. This, then, imposed a fast work of exact identification of the animal upon the second author.

Before going further, the authors want to present their sincere thanks to Mr. Fujimoto for his kindness in submitting so generously the interesting specimens to them.

Clavelina miniata n. sp.

(Figs. 1-3)

Many individuals grow densely aggregated, but they never form any social colony in a strict sense, as the individual newly formed by budding on the stolon will be separated before the opening of the branchial aperture. Thus, they may be said solitary.

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2) Contributions from the Shimoda Marine Biological Station, No. 256 and also Contributions from the Seto Marine Biological Laboratory, No. 581.

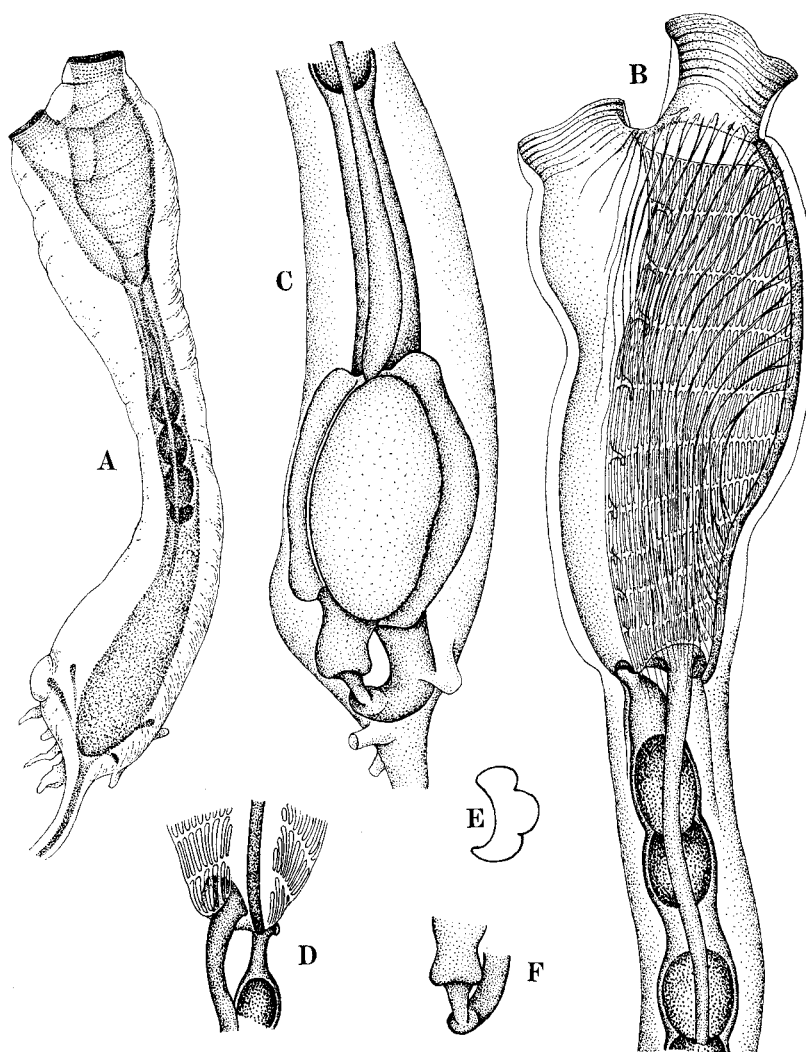


Fig. 1. *Clavelina miniata* n. sp. A: whole animal, right side. B: thorax and anterior half of abdomen, right side. C: posterior half of abdomen, right side. D: posterior end of branchial sac, ventral side. E: section of stomach. F: hind-stomach and mid-intestine.

The body is elongate, about 10 mm or slightly more in length in an extended state and consists of the thorax and abdomen; the former occupies about distal one third of the body, while the latter occupies the basal two thirds of it in an excellently fixed specimen. In living specimens, however, the thorax is 3.3–4.0 mm long and the abdomen is a little longer, 4.0–4.5 mm. Generally a single or two stolons are issued from the posterior end of the abdomen, in addition several short vascular vessels are found inside the test, but without forming any test protuberances. The animal is attached to the

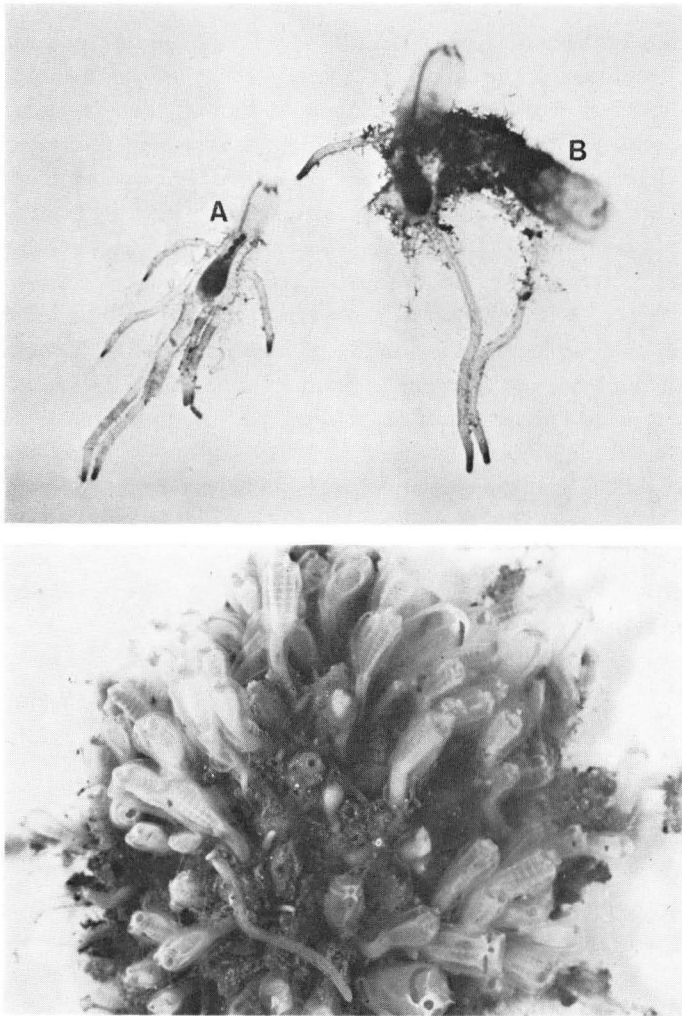


Fig. 2. *Clavelia miniata* n. sp. Top, 3 blastozooids growing on a glass-plate. Blastozooid (A) is 14 days old after the separation from its parent (B), $\times 2.2$. Bottom, a colony propagated on a glass-plate in about 240 days after it was moved there from the field, $\times 2.3$.

substratum by the left side of the posterior half of the abdomen, which is usually full of deep orange or vermillion mesenchymal cells and becomes quite opaque. A few to several short prominences may be seen along the margin of the side of attachment around the base of the stolon, but they are not related with the inner vascular vessels.

The test is gelatinous, of a considerable hardness, colourless and quite transparent so that the mantle body is wholly seen through it. The test surface is smooth and completely exposed, though some hydrozoan stolons or *Diplosoma* colonies may be growing along the test margin of the side of attachment; the stolon is exposed, too, but

it may be partly encrusted by *Diplosoma* colonies. No differentiation is seen between the thoracic and abdominal tests. In addition to the orange posterior half of the abdomen, the distal part of the stolon is coloured orange and so, but much less deeply, is around the junction between the thorax and abdomen; the endostyle and the small area between both siphons are coloured orange and somewhat fluorescent.

Thorax: Both apertures are each open on a short siphon furnished with 6–7 fine sphincters and margined smoothly, without forming any lobes; the branchial is terminal and the atrial subterminal. The thoracic musculature consists mainly of 15–20 longitudinal muscles, of which 7–9 ones are rather thicker and some dorsal ones are much thinner than others. Anterior ends of these muscles are distributed from around the base of the branchial siphon to the middle of the endostyle. Thoracic muscles pass onto the abdomen forming a thick muscle band on either side. There are 10 stigmatal rows in the largest individuals, stigmata may be up to 35 on each side in the largest rows around the middle of the branchial sac. Two to three or 4 to 5 ventral stigmata decrease the height towards the endostyle. The blastozooids just isolated are provided with (7)–8 rows of stigmata. Dorsal languets are situated nearly on the mid-dorsal line; the 7th and 8th are defined insignificantly, while the 9th is indiscernible. The prebranchial area is rather prominent. Four larger, four intermediate and 8 smaller tentacles are arranged very regularly, with a pair of larger ones on the sagittal plane; in addition a minute one may occur irregularly in some intervals in the dorsal semicircle. The ciliated groove is a very small oval opening. The oesophagus leaves the right side of the branchial sac just near its posterior end (Fig. 1D). The anus opens at the dorso-posterior corner of the thorax, not on the dorso-median line, but slightly dislocated to the left side, and is compressed dorso-ventrally.

Abdomen: The oesophagus is very elongate, the posterior end entering the stomach is much narrowed. The stomach, hind-stomach and mid-intestine are wholly buried in compactly aggregated mesenchymal cells or trophocytes and invisible unless the cells are removed under a microscope. The stomach is one fourth to one fifth as long as the abdomen, with the cardiac margin at the level of the posterior one third of the abdomen. It is concave on the left side to hold the intestine along it, while on the right side it is convex and marked with three remarkable folds (Fig. 1 C,E). The hind-stomach is a little less than a half of the stomach in length. The mid-intestine is shorter than the hind-stomach and passes to the intestine at the posterior end of the abdomen. The heart occupies the ventral side at the posterior end of the abdomen. Five to seven stolons may be seen issued from the posterior end of the abdomen in younger individuals, but these are reduced to one or two of 6–20 mm long in grown-ups. No gonads have been observed.

Remarks: The following ten species of the subfamily Clavelininae have been reported from the North West Pacific.

1. *Podoclavella molluccensis* Sluiter, 1904 Siboga-area and Philippines. (Described in 1895 as *Podoclavella meridionalis* Herdman)
2. *Dendroclavella elegans* Oka, 1927 Tateyama and Sagami Bay in Japan.

3. *Clavelina fasciculata* Van Name, 1945 Sagami Bay in Japan.
4. *Clavelina* (*Synclavella*) *arafurensis* Tokioka, 1952 Palao Islands.
5. *Podoclavella detorta* Sluiter, 1904 Siboga-area and Philippines.
6. *Clavelina coerulea* Oka, 1934 Kagosima in Japan.
7. *Clavelina minuta* Tokioka, 1962 Sagami Bay in Japan.
8. *Podoclavella fecunda* Sluiter, 1904 Siboga-area and Palao Islands.
9. *Podoclavella procera* Sluiter, 1904 Siboga-area.
10. *Podoclavella polycitorella* Tokioka, 1954 Tokara Islands in Japan.

Of these, the species from 1 to 4 form the colony more or less by fusion of individual test and then can be separated clearly from those, from 5 to 10, which are isolated or in a so-called social state. In *Podoclavella detorta*, *Clavelina coerulea* and *Clavelina minuta* zooids are connected one another by creeping and branching stolons and thus in a social state. *Podoclavella fecunda* and *P. procera* are probably solitary and *P. polycitorella* is distinctly so. *P. fecunda* is larger, up to 18 mm in length in a preserved state; the stomach surface is smooth; and there are up to 40 tentacles inclusive of larger, intermediate and smaller ones. *P. procera* is much larger, attaining to 80 mm in length, and has a very elongate stalk supporting the animal body; the anus opens at the level of the middle of the branchial sac; and tentacles are arranged in two rings. *P. polycitorella* attaches to the substratum nearly along the whole body and has the abdomen much shorter than in the present form; the stomach wall is quite smooth.

The morphology of the present form resembles rather closely that of the typical species of *Clavelina*, *C. lepadiformis* (Müller), but the former is separable distinctly from the latter by the solitary life type and somewhat fewer stigmatal rows. The present form may safely be treated as a new species which is here named by both authors *Clavelina miniata* for the abdomen that is coloured deeply by aggregated vermilion mesenchymal cells.

Asexual Reproduction

The budding manner of this new species is septal mesenchymal and fundamentally the same as in other species of *Clavelina*. The budding process from blastozooids growing on the glass-plates had been observed repeatedly and it was found that the process was perfectly uniform. Therefore, the following observation of the process made in last September to October on a certain group of specimens may be given as a general explanation of the phenomenon.

In all cases, prior to the bud-formation, large cells pigmented orange and therefore called "orange cells" hereafter, which had been deposited around the endostyle, between both siphons, and in the posterior half of the abdomen, migrated into the stolon vessel extending from the posterior end of the body and rapidly accumulated densely on the side-wall of the vessel, on the septum separating the afferent and efferent flows of the blood in the stolon, or in the terminal part of the vessel (September 29, 09 : 00, Fig. 3 a).

In 5 to 6 hours after the accumulation of these "orange cells," a bud-rudiment of about 3.0 mm long became definable in a certain mass of "orange cells" when it had been very densely deposited in roughly 8.0–11.0 mm from the tip of the stolon vessel which was about 17.0 mm in length. At the same time, the portion of the stolon (ca. 5.4 mm long) distal to the bud-rudiment began to diminish the length gradually, though the circulation of blood corpuscles in the distal part was maintained steadily. On the other hand, the stolon vessel connecting the bud to the parental zooid became thinner and thinner to a connecting thread of about $50\ \mu$ in thickness (Fig. 3 b), which then broke within the test and thus the empty test of the stolon was left connecting the bud to the parent (Fig. 3 c). The morphogenesis in the bud usually began strictly after the isolation from the parental zooid as in other forms of *Clavelina* (Berrill, 1935). The first of all, externally observable, was the appearance of a clear area, in which the heart was beating at the postero-ventral part of the initial bud (September 29, 15 : 00, Fig. 3 b); thus the heart is the internal organ which will become functional first.

Soon after the internal separation, the bud itself shortened to about a half of its original length (ca. 1.6 mm) and sometimes became roundish in shape, this might be due to epidermal cells becoming taller to be columnar. Soon later, the thoracic rudiment began to inflate, extending anteriorly, to form a translucent part of about 0.5 mm long. However, nothing else than the heart-beat could be seen exactly within the bud, as it was so opaque. On the other hand, three stolons started newly to protrude from the posterior part of the bud, in addition to the reduced original stolon (September 30, 14 : 30, Fig. 3 c).

The bud passed then 3 or 4 days for elongation of the body before it began to feed. The stolon processes grew out on the substratum in irregular directions from the basal region of or around the abdomen (October 1, 15 : 00, Fig. 3 d).

The bud, together with its stolon processes, continued to grow till the siphons slightly opened on it still within the thin envelope of the test of the parental stolon. This envelope binding the bud to the parent gradually torn, and finally the bud and the parent were completely separated from each other (October 2, 15 : 00, Fig. 3 e; October 3, 14 : 50, Fig. 3 f).

Thus, in about 5 days after the isolation from the parent the apertures opened on the bud and the new individual became fully functional, starting feeding. The thorax and abdomen of such a new functional zooid were 1.8 mm and 2.7 mm long respectively (October 4, 14 : 06, Fig. 3 g). The above-mentioned process went on at the water temperature of 21–23°C. Further development included the gradual reduction of stolon processes, but leaving one or two long vessels.

Generally speaking, shortly before the separation of the bud from the parental zooid, a high concentration of "orange cells" will be recognized at a position in a certain distance to the tip of the large stolon extending from the posterior end of the parent abdomen. The first functional structure appearing in this region of the stolon is the heart which is situated on the postero-ventral side of the bud. The development of the heart immediately after the separation of the bud from the parental zooid is

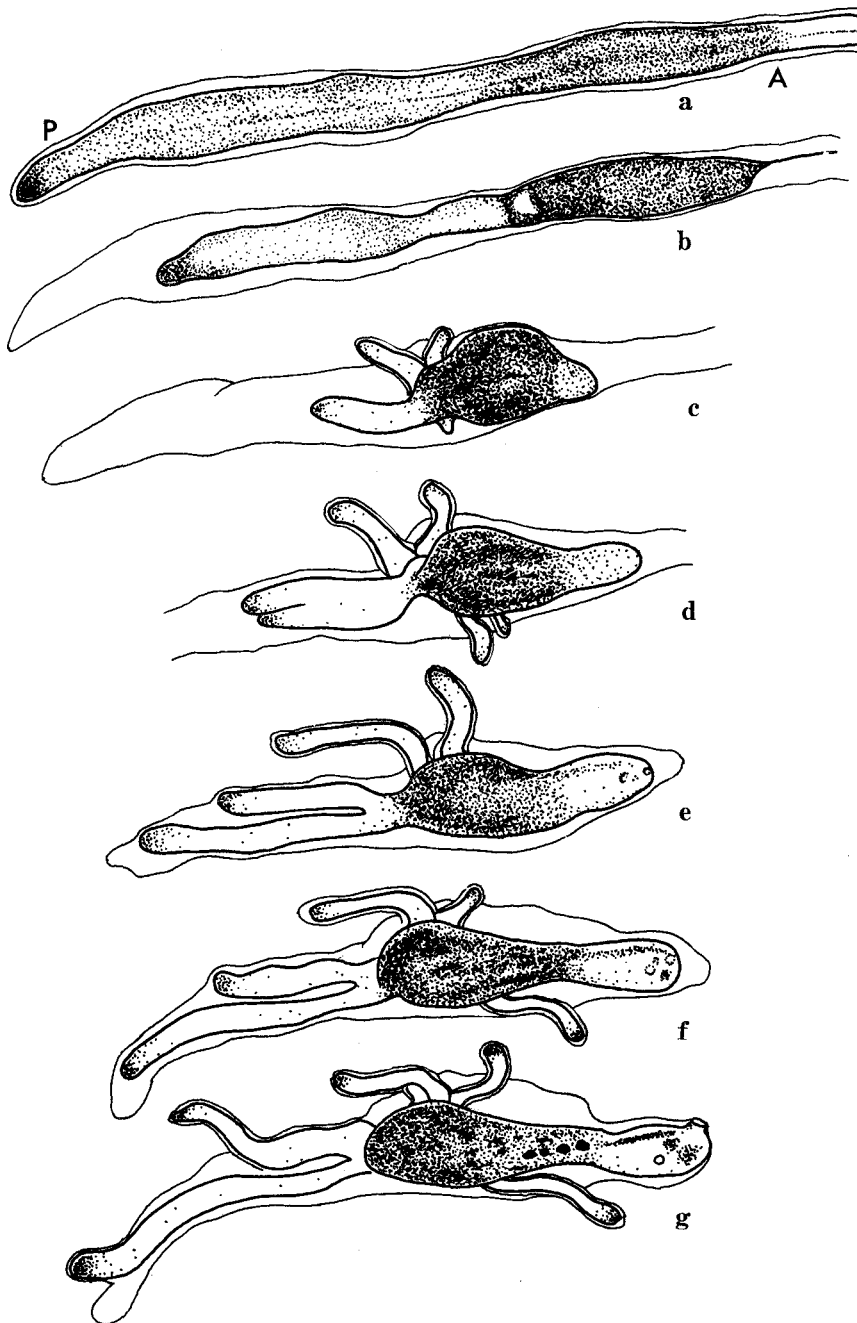


Fig. 3. Successive processes of budding from a blastozoid in *Clavelina miniata* n. sp. (a-g), $\times 12.3$. A, anterior; P, posterior.

general throughout any developing buds in the present new species. Not a single case has been recorded in which the bud is formed at the tip of the stolon vessel, when "orange cells" are usually densely deposited there.

During the entire process of budding, the parental zooid maintains continuously its functional pharynx. According to Berrill (1935), the sexual reproduction in species of the subfamily Clavelininae takes place, as a rule, during the summer months. At the end of the season, adult zooids become degenerate or are sloughed off, leaving the congested posterior stolon processes to give rise to new buds during the winter. Then, the asexual reproduction occurs during the winter months. It is noteworthy that the resorption or disappearance (sloughing off) of the parental zooid never occurs in the present new species throughout the year after issuing buds, though the season for its sexual reproduction is still unknown (Fig. 2).

Immediately after the isolation of the bud, newly formed stolon processes may number 5 to 7, excluding the original stolon, though later lesser vessels are usually withdrawn, leaving only 1 or 2 prominent ones (Fig. 2). They sometimes display positive geotropism.

In the majority of specimens observed, the buds grew into a feeding blastozooid congested with "orange cells" within 5 to 6 days after they were isolated. Newly developed blastozooid starts to produce its own bud 3 or 4 weeks after its commencement of feeding in the same manner as that just described. The blastozooids at this stage measure 3.8–4.2 mm in abdominal length. As far as the observations are concerned, the polarity of the bud individual is kept usually the same as that of the parent, but only in one case, in which the polarity was reversed in the bud individual.

Very probably, the above-mentioned manner of budding is actually going on in the present new species in the natural environments.

In *Amaroucium constellatum*, the activities of cells in the dorsal half of the thorax normally regulate the strobilization, one kind of asexual reproduction in tunicates (Freeman, 1971). In the present new species, budding is always initiated by the accumulation of "orange cells" in a certain distance from the tip of stolon vessels. However, this accumulation takes place too rapidly to be observed in detail. At present, nothing is known of the internal mechanism how the accumulation of "orange cells" is regulated in the present new species. To answer this question, further investigations on intrinsic factors, as stated by Freeman, which regulate the initiation of asexual reproduction in the present species will be needed.

In *Pycnoclavella stanleyi* belonging to the subfamily Clavelininae, budding is effected by the formation of a single transverse epidermal constriction anterior to the stomach in the abdomen, accompanied by the formation of a stolon process, in which the cells containing orange pigments appear to play some role in budding. This is more similar to forms found in the Polycitorinae than to those found in the Clavelininae (Trason, 1963). Also in a still undescribed species of *Clavelina* occurring in the vicinity of the Friday Harbor Laboratories, it was found (though unpublished) that

the formation of buds was initiated by transverse epidermal constriction at the similar level in the abdomen as in *Pycnoclavella*. Thus in these forms, the epicardium running along the ventral side of the abdomen is the main formative tissue in the regeneration of new structures as in forms of the Polycitorinae.

Taking the above-mentioned type of budding found in the present new species in consideration, it seems inevitable that the old conception of the mode of budding in the Clavelininae, one of the three subfamilies of the family Polycitoridae (Berrill, 1950), should be revised by checking on respective species.

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